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The Expression of NAD(P)H:Quinone Oxidoreductase 1 Is High in Human Adipose Tissue, Reduced by Weight Loss, and Correlates with Adiposity, Insulin Sensitivity, and Markers of Liver Dysfunction

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Context: We have previously identified nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase 1 (NQO1), an enzyme involved in the protection against oxidative stress, as a gene predominantly expressed in human adipocytes. Studies in mice deficient in NQO1 activity suggest that NQO1 may also play an important role in metabolism.

Objective: The aim of this study was to explore the expression and regulation of NQO1 in human adipose tissue (AT) and isolated adipocytes.

Patients and Results: The high expression of NQO1 in adipocytes was verified in human adipocytes and AT by real-time PCR. DNA microarray analysis showed that NQO1 was expressed at higher levels in large compared with small adipocytes, isolated from the same fat biopsy. Furthermore, NQO1 mRNA levels were positively correlated with adipocyte size (n = 7; $P < 0.002$). During an 18-wk diet regime (n = 24; mean weight loss 27 kg), the NQO1 expression in human sc AT was down-regulated ($P < 0.0001$), and mRNA levels correlated with body mass index ($P = 0.0005$), sc, and total abdominal AT areas, as determined by computerized tomography ($P < 0.0001$, both) and metabolic parameters. NQO1 mRNA levels were also positively correlated with aspartate aminotransferase ($P = 0.0028$) and alanine aminotransferase ($P = 0.0219$), markers known to be associated with severity of hepatic steatosis.

Conclusions: NQO1 is highly expressed in human AT, particularly in large adipocytes. AT NQO1 expression is reduced during diet-induced weight loss, and the expression levels positively correlate with adiposity, glucose tolerance, and markers of liver dysfunction. Together, these findings indicate a role for NQO1 in the metabolic complications of human obesity. **(*J Clin Endocrinol Metab* 92: 2346–2352, 2007)**

NICOTINAMIDE ADENINE dinucleotide phosphate: quinone oxidoreductase 1 (NQO1) is part of the antioxidant defense system, and has been extensively studied in relation to chemoprotection, cancer susceptibility, and antitumor agents (1). However, studies in mice deficient in NQO1 activity suggest that NQO1 also plays an important role in metabolism. The adult NQO1 knockout mice have markedly reduced abdominal adipose tissue (AT) mass, higher levels of triglycerides (TGs), and lower levels of glucose in the blood compared with wild-type mice. In addition, $NQO1^{-/-}$ mice have higher TG content in the liver compared with wild-type mice (2).

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Abbreviations: ALT, Alanine aminotransferase; AST, aspartate aminotransferase; AT, adipose tissue; BMI, body mass index; CT, computerized tomography; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MRS, magnetic resonance spectroscopy; NQO1, quinone oxidoreductase 1; OGTT, oral glucose tolerance test; PPIA, peptidylprolyl isomerase A; SNP, single nucleotide polymorphism; SOS, Swedish obese subjects; TG, triglyceride; VLCD, very low-calorie diet; WHR, waist-to-hip ratio.

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It is well known that NQO1 is involved in the body's defense system against oxidative stress (1, 2). The enzymatic function of NQO1 is to catalyze the detoxification of quinones and their derivatives, preventing their participation in redox cycling and oxidative stress (2, 3). NQO1 has also attracted attention as a flavoenzyme associated with protection against mutagenesis and carcinogenesis (3, 4). A C609T substitution of the NQO1 gene, which contributes to an amino acid change, results in loss of NQO1 activity in humans (5). This polymorphism varies (range 4–22%) among ethnic groups (1).

NQO1 expression has been detected in many human tissues, and large variation in NQO1 expression and activity has been found (3). Although NQO1 expression has been reported in AT (2, 6) and adipocytes (7), we were surprised to find that NQO1 expression was most abundant in human adipocytes when performing a DNA microarray screen for adipocyte-specific genes (8, 9). The high expression in human adipocytes and the striking effects of NQO1 deficiency on abdominal AT mass in mice suggest a possible role for NQO1 in energy homeostasis and adipocyte function. Consequently, studies on this gene in humans may provide important information on the development of obesity and obesity associated metabolic disease. Because knowledge about

the regulation of NQO1 expression in human AT is limited, the aim of this study was to analyze possible expression changes during diet-induced weight loss in obese subjects, possible correlations with parameters associated with metabolic complications of obesity, as well as to examine the expression of this gene in different human tissues, fat depots, and in adipocytes of different size. We also wanted to study if the previously identified C609T polymorphism in the NQO1 gene is associated with obesity and metabolic parameters.

Subjects and Methods

Subjects and samples

Obese subjects were treated with a very low-calorie diet (VLCD) (450 kcal/d, Cambridge diet or Modifast) for 16 wk, before a normal diet was gradually reintroduced. After 16 wk, one VLCD meal a day is replaced by an ordinary meal, and after 17 wk, two meals are replaced by ordinary meals. During wk 17 and 18, the body weight is stabilized. Abdominal sc AT biopsies were obtained before (wk 0), during (wk 8 and 16), and after (wk 18) diet-induced weight loss from 24 obese subjects (6 women and 18 men), and used for expression level analysis by DNA microarray (Table 1). Subjects analyzed by DNA microarray displayed a mean weight loss of 27 kg after 18 wk of diet. Abdominal sc AT biopsies were also obtained before (wk 0), during (wk 8), and after (wk 18) diet-induced weight loss from eight healthy obese men [baseline body mass index (BMI) 37 ± 3 kg/m^2, mean weight loss 19 ± 9 kg] and used for expression level analysis by real-time PCR. For isolation and separation of small and large adipocytes, sc AT was obtained from six women (BMI 24 ± 2 kg/m^2) and three men (BMI 27 ± 1 kg/m^2) (10). In brief, adipocytes were isolated by collagenase treatment (11). The cell suspension was gently agitated, and cells that resurfaced within 30 sec were transferred to new tubes; this procedure was repeated once. These more buoyant cells were filtered through a nylon mesh (70 μm), and cells not passing through were considered the final preparation of large adipocytes. The adipocytes that did not resurface within 30 sec were filtered through a nylon mesh (50 μm), and cells passing through were considered the final preparation of small adipocytes (10). To study depot and gender differences, AT biopsies from the abdominal sc depot and the major omentum were obtained from seven obese men (BMI 42 ± 5 kg/m^2) (12) and four obese women (BMI 54 ± 13 kg/m^2) (8) undergoing laparoscopic bariatric surgery. Analysis of NQO1 expression in adipocytes and the stromal-vascular fraction from AT was performed in four obese subjects (BMI 43 ± 6 kg/m^2). Analysis of NQO1 expression in individuals with different BMI was performed in 12 men and 12 women (obese n = 12, BMI range 33.2–51.4 kg/m^2; overweight, n = 8, BMI range 25.0–29.0 kg/m^2; lean, n = 4, BMI range 23.0–24.9 kg/m^2). For genotype analysis, DNA was isolated from blood samples from the Swedish obese subjects (SOS) reference study (13) (189 lean, 131 healthy obese, and 250 dysmetabolic obese subjects) according to the adult treatment panel III (14). For verification, two additional populations were used: the (INTERGENE) case-control study (642 subjects with coronary artery disease and 642 healthy controls) (15); and the Dallas Heart Study (16, 17) (1830 African-Americans, 601 Hispanics, and 1045 European Americans). Regional Ethical Review Boards approved the studies, and all participants gave written informed consent. Characteristics of the cohorts used for genotyping are shown in Table 2.

RNA and DNA samples, and DNA microarray expression profiles

RNA was isolated as described by Chomczynski and Sacchi (18), and further purified with RNeasy clean-up columns (QIAGEN, Hilden, Germany) or using the RNeasy Lipid Tissue Kit (QIAGEN). The gene expression in AT depots, AT during diet-induced weight loss, and in adipocytes of different sizes have previously been analyzed using DNA microarray (Affymetrix, Santa Clara, CA) (8, 10, 12). Real-time PCR was used to confirm the results. For verification of the gene expression in different human tissues by real-time PCR, RNA from the Human Total RNA Master Panel II (CLONTECH, Palo Alto, CA) was used. Genomic DNA was isolated using a standard proteinase K DNA isolation protocol, FlexiGene DNA kit (QIAGEN), or Pure Gene (Gentra Systems, Minneapolis, MN).

TABLE 1. Characteristics of the patients included in the VLCD study and association of NQO1 expression, analyzed by DNA microarray, with anthropometric measurements and metabolic parameters during diet-induced weight loss

	Week				Statistics			
					Nonadjusted		BMI adjusted	
	0	8	16	18	Slope	*P* value	Slope	*P* value
BMI (kg/m^2)	37.6 ± 4.9	31.8 ± 4.1	28.6 ± 4.1	28.9 ± 3.9	1.56	0.0005		
WHR	1.02 ± 0.08	0.99 ± 0.08	0.95 ± 0.08	0.95 ± 0.08	1.86	0.0766	−0.12	0.9380
TAT (cm^2)	778 ± 191		419 ± 174		0.82	<0.0001	0.85	0.0024
sc AT (cm^2)	526 ± 166		311 ± 137		0.71	<0.0001	0.65	0.0018
VAT (cm^2)	241 ± 76		101 ± 49		0.40	0.0577	0.11	0.6501
Liver attenuation (HU + 1000)	1042 ± 13.5		1051 ± 7.2		−8.36	0.2884	2.08	0.7431
Fasting glucose (mmol/liter)	6.0 ± 1.6	4.5 ± 0.7	4.5 ± 0.7	5.0 ± 1.0	0.65	0.0683	0.47	0.0258
Fasting insulin (mU/liter)	15.8 ± 7.4	7.0 ± 4.1	4.3 ± 2.2	6.3 ± 3.7	0.27	0.0303	0.11	0.4126
OGTT glucose (mmol/liter)	8.2 ± 3.8	7.0 ± 1.9	7.0 ± 2.6	5.9 ± 2.3	0.38	0.0164	0.34	0.0081
OGTT insulin (mU/liter)	66.7 ± 42.9	41.4 ± 20.7	34.0 ± 16.6	24.1 ± 9.9	0.20	0.0194	0.14	0.0795
HOMA	4.4 ± 2.7	1.4 ± 0.9	0.9 ± 0.5	1.5 ± 1.3	0.25	0.0154	0.12	0.1929
TG (mmol/liter)	1.8 ± 1.0	1.0 ± 0.2	0.9 ± 0.2	1.2 ± 0.5	0.13	0.4709	−0.09	0.5142
HDL (mmol/liter)	1.4 ± 0.4	1.2 ± 0.3	1.4 ± 0.4	1.4 ± 0.3	0.33	0.1884	0.55	0.0185
LDL (mmol/liter)	3.6 ± 1.0	2.3 ± 0.8	2.6 ± 0.6	2.9 ± 0.7	0.07	0.7276	0.17	0.2307
AST (μkat/liter)	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.1	0.55	0.0028	0.47	0.0048
ALT (μkat/liter)	0.7 ± 0.3	0.9 ± 0.7	0.5 ± 0.3	0.5 ± 0.3	0.25	0.0219	0.16	0.1099

Serum samples were obtained before, during, and after VLCD. CT performed at wk 0 and 16 were used for adipose tissue area and liver attenuation calculations. Glucose, insulin, OGTT glucose, OGTT insulin, TG, HDL, LDL, AST, and ALT were measured during VLCD. The slope is the estimated regression coefficient between the log of the predictor variable and the log of NQO1 gene expression. A *P* value for the test that the slope was equal to zero during the diet was calculated for each parameter. Two values for slope and *P* value are given for each predictor, nonadjusted and adjusted for changes in BMI during diet-induced weight loss. Of the 24 patients included, 12 were obese but healthy, and 12 were dysmetabolic according to the criteria set by the World Health Organization (14); 6 women and 18 men except TAT/SAT/VAT (n = 23) wk 16. Note that a constant was added to liver attenuation values to avoid theoretical negative values, which cannot be log transformed. HOMA, Homeostasis model of assessment; HU, Hounsfield unit; TAT, total adipose tissue; VAT, visceral adipose tissue.

TABLE 2. Characteristics of the patients used for the genotyping studies

Population	SOS reference study (13)			INTERGENE (15)		Dallas Heart Study (16, 17)		
	Healthy obese	Dysmetabolic obese	Lean	Case	Control	African-American	Hispanics	European Americans
No. of subjects	131	250	189	642	642	1830	601	1045
Male to female ratio	47:84	79:171	54:135	456:186	456:186	773:1057	251:350	500:545
Mean age at examination ± SD	45.7 ± 6.6 yr	46.4 ± 6.5 yr	46.9 ± 6.3 yr	61.7 ± 8.3 yr	61.8 ± 8.3 yr	45.6 ± 10 yr	40.9 ± 9 yr	45.3 ± 10 yr
Mean BMI ± SD	38.1 ± 3.8 kg/m^2	38.3 ± 3.6 kg/m^2	21.9 ± 1.5 kg/m^2	27.9 ± 4.2 kg/m^2	26.9 ± 3.7 kg/m^2	31.9 ± 8 kg/m^2	30.6 ± 7 kg/m^2	29.1 ± 7 kg/m^2

Measurements

Computerized tomography (CT) was used to determine body composition before (wk 0) and during (wk 16) the VLCD treatment, as previously described (12). CT or proton nuclear magnetic resonance spectroscopy (MRS) (16) was used to determine hepatic fat content using attenuation and signal intensity values, respectively. Fasting glucose and insulin, oral glucose tolerance test (OGTT) glucose and insulin, TG, high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were analyzed at the Department of Clinical Chemistry, Sahlgrenska University Hospital (accredited according to the international standard ISO/IEC 17025).

Real-time PCR analysis

Reagents for real-time PCR analysis of NQO1 and peptidyl-prolyl isomerase A (PPIA) (cyclophilin A) (Assays-on-Demand, TaqMan Reverse Transcriptase reagents and TaqMan Universal PCR Master mix) were purchased from Applied Biosystems (Foster City, CA) and used according to the manufacturer's protocol. cDNA synthesis was performed in a total volume of 50 μl using 500-ng total RNA. cDNA corresponding to 10 ng RNA per reaction was used for real-time PCR amplification and detection in the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) using default cycle parameters. A standard curve was plotted for each primer-probe set with a serial dilution of pooled AT cDNA. Human PPIA was used as a reference gene.

Genotyping

Primers and probes (primers; forward: 5′–GGGCGTCTGCTGGAGTGT, reverse: 5′–CCTCAGAGTGGCATTCTGCAT and probes; 5′ VIC–ATGTCAGTTGAGGTTC–MGB, 5′ FAM–ATGTCAGTTGAGATTC–MGB) for the NQO1 polymorphism C609T (rs1800566) were designed by Applied Biosystems. Primers, probes and TaqMan Universal PCR Master mix were purchased from Applied Biosystems and used according to the manufacturer's protocol. The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) was used for amplification and detection.

Statistical analysis

Values are expressed as mean ± SD unless otherwise stated. Difference in NQO1 expression between small and large adipocytes was analyzed using the Wilcoxon signed rank test. Difference in NQO1 expression between adipocyte and the stromal-vascular fraction was analyzed using the Mann-Whitney *U* test. The relationship between NQO1 gene expression and adipocyte size, as well as NQO1 expression and BMI was analyzed using the Spearman rank correlation test. Differences between TG levels and waist-to-hip ratio (WHR) by NQO1 genotype in the SOS reference study were analyzed using Student's *t* test. Kruskal-Wallis or ANOVA was used for genotype association analysis in the INTERGENE and Dallas Heart Study populations.

The analyses of NQO1 gene expression, presented in Table 1, were initially performed with standard linear regression. Correlation of within-person longitudinal measurements was addressed with the use of generalized estimating equations (19). Tests of parameters within these models were performed with generalized Wald tests (20), which yielded standard Z statistics. We fit a common slope across the different weeks, which is presented in Table 1, but allowed for different intercept terms corresponding to week-specific levels of NQO1. Because the data were skewed, logarithmic transformations were applied to the various NQO1 variables and predictors. An exact binomial test was used to assess down-regulation. Down-regulation will be significant if most subjects have the highest expression level at wk 0. If the expression levels are equal across the weeks, no down-regulation will be detected. We used an α of 0.05 to assess significance of the various tests.

Results

Expression of NQO1 in human tissues and AT depots

In previous studies we searched for genes that are predominantly expressed in human adipocytes using gene expression profiles generated by DNA microarray from adipocytes, and 32 other human tissues and cell types (8, 9). We showed that NQO1 was highly expressed in both human omental and sc adipocytes. The DNA microarray data indi-

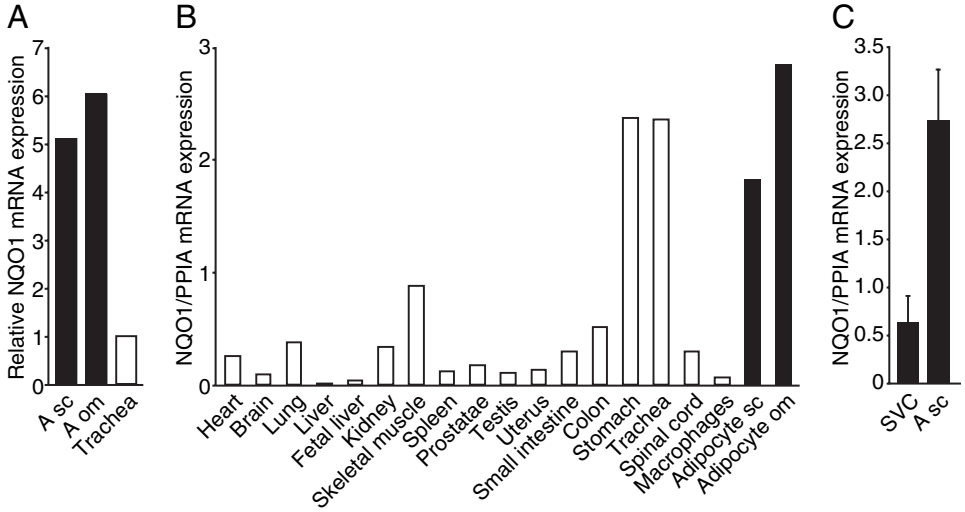

FIG. 1. The relative expression of NQO1 as analyzed by U95A DNA microarray in sc (A sc) and omental adipocytes (A om), as well as trachea, the tissue with the second highest expression (A). Values correspond to the 38066_at probe set. NQO1 expression in a panel of 17 human tissues and cell types in addition to sc and omental adipocytes (*black bars*) analyzed by real-time PCR (B). NQO1 expression (mean ± SEM) in human adipocytes and stromal-vascular cells (SVC) (C).

cated that NQO1 expression was highest in adipocytes and that trachea was the tissue with the second highest expression (Fig. 1A). To verify the results, we measured NQO1 expression by real-time PCR in human adipocytes and in a panel of 17 human tissues (Fig. 1B). The results confirmed the high expression in omental and sc adipocytes, although trachea and stomach had similar expression levels. To verify that NQO1 expression originates in the adipocyte fraction of AT, real-time PCR analysis on RNA from adipocytes and the stromal-vascular fraction was performed in four subjects. The results showed that adipocytes expressed over 4 times higher levels of NQO1 mRNA (Fig. 1C; $P < 0.016$). When performing the adipocyte isolation procedure, the stromal-vascular fraction will not be completely free from adipocytes. Therefore, contaminating adipocytes may be the reason for detectable levels in this fraction. We also compared NQO1 expression in paired AT biopsies from the sc and omental depots. However, the expression did not differ significantly between the two depots or between genders (data not shown).

Expression of NQO1 in lean, overweight, and obese subjects

RNA was isolated from AT biopsies from 12 obese, eight overweight, and four lean individuals (Fig. 2). The NQO1 expression was clearly correlated with BMI ($r = 0.582$; $P = 0.03$).

Expression of NQO1 in small and large adipocytes

Adipocytes isolated from human ATs biopsies were separated into populations of small and large cells (10). The NQO1 expression was 3.7-fold higher in large (99.4 ± 13.8 μm) compared with small (60.7 ± 10.7 μm) adipocytes in three paired samples analyzed by DNA microarray (10). The higher expression in large adipocytes was confirmed in adipocyte populations from seven subjects using real-time PCR. In all cases, NQO1 was expressed at higher levels in the large adipocytes ($P = 0.0176$), with a mean fold increase of 4.4 ± 3.2 compared with the expression in small adipocytes. In addition, there was a positive correlation between the NQO1 gene expression and adipocyte diameter ($r = 0.763$; $P = 0.002$; Fig. 3).

Effect of weight loss on NQO1 expression in sc AT

To study the effects of weight loss, NQO1 mRNA expression was analyzed in sc AT from obese subjects before (wk 0), during (wk 8 and/or 16), and after (wk 18) VLCD treatment. The NQO1 mRNA levels were reduced during the diet-induced weight loss according to both the DNA microarray ($P < 0.0001$; Fig. 4A) and real-time PCR ($P = 0.002$; Fig. 4B) data.

Association of NQO1 expression with anthropometric measurements and metabolic parameters during diet-induced weight loss

Our observation that NQO1 is highly expressed in adipocytes, together with the down-regulation of NQO1 expression during diet-induced weight loss, led us to examine correlations between NQO1 expression and body composition, as well as metabolic parameters. These results are summarized in Table 1. The NQO1 mRNA levels positively correlated with total and sc abdominal AT area, as well as BMI during diet-induced weight loss. The NQO1 expression was also positively correlated with fasting insulin, as well as to levels of insulin and glucose during OGTT. NQO1 expression levels did not correlate significantly with levels of HDL, LDL, or TG. After adjustment for changes in BMI, NQO1 expression levels remained correlated with fasting levels of glucose as well as OGTT glucose and insulin, but not to fasting levels of insulin (Table 1).

NQO1 expression and hepatic function during diet-induced weight loss

It is well established that obesity is associated with the deposition of TG in the liver and nonalcoholic fatty liver disease (21). In mice deficient in NQO1, the TG concentration in the liver is markedly increased (2). Excessive TG accu-

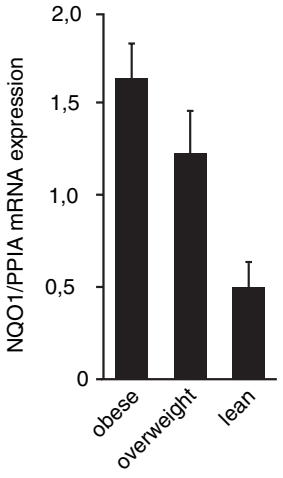

FIG. 2. The expression of NQO1 (mean ± SEM) in AT from obese (n = 12), overweight (n = 8), and lean (n = 4) subjects. BMI was correlated with NQO1 expression ($P = 0.003$).

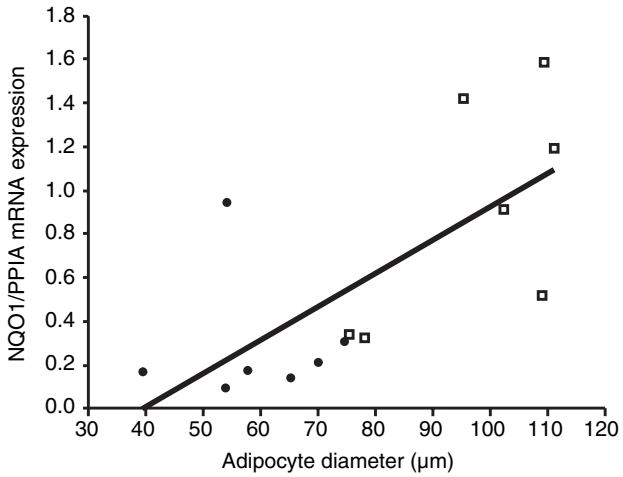

FIG. 3. NQO1 gene expression in small and large human adipocytes, isolated from a single biopsy (n = 7). *Closed circles* represent small adipocytes and *open boxes* large adipocytes. NQO1 gene expression was correlated with adipocyte size when analyzed by real-time PCR ($P = 0.002$).

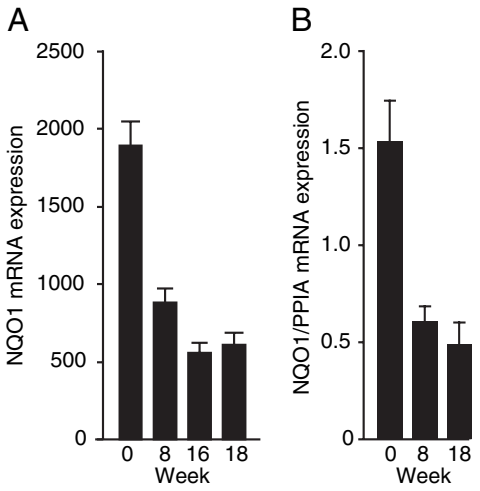

FIG. 4. Effect of diet-induced weight loss on NQO1 gene expression in sc AT during VLCD. mRNA expression levels analyzed by U133A DNA microarray (A). Values correspond to the 201468_s_at probe set. The NQO1 expression was significantly down-regulated during the diet-induced weight loss ($P < 0.0001$; n = 24). The down-regulation of NQO1 expression was confirmed using real-time PCR ($P = 0.002$; n = 8), mean ± SEM (B). Note that NQO1 expression was significantly down-regulated both between baseline and 8 wk ($P < 0.0001$), and between 8 and 16 wk ($P = 0.0017$) when analyzed by DNA microarray.

mulation in the liver has recently been the focus as a mediator of the metabolic complications seen in obese subjects (22). Therefore, we studied NQO1 expression in relation to markers of hepatic function in the subjects treated with VLCD. Positive correlations were seen between NQO1 expression and two markers of liver dysfunction (23): AST ($P = 0.0028$) and ALT ($P = 0.0219$) (Table 1). However, no correlation was seen between NQO1 expression and liver attenuation analyzed by CT ($P = 0.2884$). After adjustment for changes in BMI during diet-induced weight loss, NQO1 expression levels were correlated with AST levels, but not with levels of ALT (Table 1).

Associations between the NQO1 single nucleotide polymorphism (SNP) and metabolic parameters

A SNP in the coding region of NQO1 has previously been characterized (5). The polymorphism is a C to T substitution at position 609 of the NQO1 gene and results in a proline to serine change at position 187 of the NQO1 protein. Individuals heterozygous for the T allele display significantly lower NQO1 protein levels, and individuals homozygous for the T allele have no detectable levels of NQO1 protein (5). In subgroups from the SOS reference study population, we found an association between the non-CC genotype and lower WHR in healthy obese subjects (n = 131; $P = 0.003$), and an association between the non-CC genotype and higher serum TG levels in obese subjects with metabolic disease (n = 250; $P = 0.049$). However, we were not able to verify the associations between genotype and the parameters mentioned previously, or in the INTERGENE or Dallas Heart Study populations, which include both obese and nonobese subjects. In the INTERGENE study, no associations were found between NQO1 genotype and BMI, WHR, blood pressure, serum glucose, cholesterol, TG, HDL, or LDL in the complete patient material. No difference in genotype frequency was found between the case and control groups. In the Dallas Heart Study, no associations were found between NQO1 genotype and BMI, AT mass, blood pressure, serum glucose, serum insulin, cholesterol, TG, HDL, LDL, or hepatic TG content in either of the ethnic groups, regardless of gender. We found a TT genotype frequency of 1.75% in the SOS reference study, 2.41% in the INTERGENE study, and 3.4%, 4.7%, and 12.4%, respectively, in the European American, African-American, and Hispanic populations included in the Dallas Heart Study. These results are comparable with the ones previously reported (1).

Discussion

Obesity is often accompanied by complications such as type 2 diabetes, hypertension, hyperlipidemia, hypercholesterolemia, and cardiovascular disease (24), as well as hepatic steatosis (21). Short-term studies on intentional weight loss have shown drastic improvement in all aspects of the metabolic syndrome (24), including improved glycemic control, decreased blood pressure, and reduced cholesterol levels. Furthermore, weight loss is associated with a decrease in liver TG content (25). In the present study we demonstrate that NQO1 is expressed at high levels in human adipocytes, and that the mRNA levels in AT during diet-induced weight loss are significantly correlated with several anthropometric measurements of obesity, AT depot size, measurements of glucose tolerance, and markers known to be associated with severity of hepatic steatosis.

It is known that adipocyte function and gene expression differ in adipocytes of different sizes (10, 26, 27). In this study all subjects analyzed had higher expression of NQO1 in large compared with small adipocytes. Enlargement of sc adipocytes is associated with insulin resistance, and the risk of metabolic complications increases with increasing adipocyte size (28). Furthermore, it has been shown that oxidative stress impairs insulin action in adipocytes *in vitro* (29) and that hyperglycemia leads to the generation of reactive oxygen species, resulting in oxidative stress in different tissues (30, 31). Dandona *et al.* (32) have reported that the oxidative damage to lipids, proteins, and amino acids is increased in obese subjects and that this damage is reduced by weight loss. However, it is not completely understood how obesity or metabolic disorders may cause oxidative stress. Whether NQO1 is a marker for oxidative stress and/or adipose dysfunction is unknown, but the data presented here indicate that enlargement of adipocytes is associated with increased oxidative stress in AT.

It has also been shown that obesity results in dysregulation of adipokines and a state of low-grade chronic inflammation (33). It is known that AT secretes a variety of inflammatory cytokines such as TNF-α and IL-6 (34), as well as acute phase proteins (8). Furthermore, AT contains many cell types other than adipocytes, such as fibroblasts, endothelial cells, leukocytes, monocytes, and macrophages, that together constitute the stromal-vascular fraction. Macrophages secrete proinflammatory cytokines, opening up the possibility for adipocyte and macrophage crosstalk (34) that may have an effect on NQO1 expression.

Several metabolic pathways are altered in NQO1 knockout mice. These mice are also suggested to be insulin resistant (2), which is somewhat surprising because reduced abdominal AT mass is usually associated with higher insulin sensitivity, at least in humans (24). However, extreme loss of AT, seen in patients who have lipodystrophy, results in insulin resistance (35). This indicates that an optimal level of AT mass gives the optimal insulin sensitivity. The knockout study suggests that NQO1 has important effects on fat storage and metabolism, at least in mice. In our study NQO1 expression in human sc AT was positively correlated with insulin levels, as well as OGTT levels of glucose and insulin. In contrast, no correlation was seen with levels of TG and glucose during diet-induced weight loss.

The human NQO1 C609T polymorphism causes variation in NQO1 protein levels (5), and NQO1 deficient mice have highly reduced abdominal AT mass (2). In line with this, we found that the non-CC genotype was associated with a lower WHR in a population of healthy obese subjects and with high TG levels in a population of dysmetabolic obese subjects. However, we were not able to find any associations in the complete study population, and we could not repeat these results in two other larger populations. Furthermore, in a recent Chinese case-control study (36) (mean BMI 25), no association was identified between the NQO1 polymorphism and type 2 diabetes.

NQO1$^{-/-}$ mice also display increased TG content in the liver (2), *i.e.* hepatic steatosis (21). Hepatic steatosis affects approximately 30 million Americans (21) and is almost always present in obese persons with high alcohol consumption. However, hepatic steatosis is more strongly associated with accumulation of excess body fat than with high alcohol intake. A recent study (37) indicates that abdominal fat accumulation, a risk factor of the metabolic syndrome, may be an independent predictor of hepatic steatosis (38). We found significant positive correlations between NQO1 mRNA levels and markers known to be associated with the severity of hepatic steatosis (ALT, AST) during diet-induced weight loss in obese subjects. However, no significant correlations were seen between NQO1 and the amount of hepatic fat in the VLCD study (expression; CT) or the Dallas Heart Study (genotype; MRS). The reason for this finding is unknown, but ALT and AST are markers for liver dysfunction, including steatosis, whereas CT (39) or, in particular, MRS (40) determination of hepatic fat content is more precise. Thus, it may be speculated that AT NQO1 expression may be related to some factor, other than fat accumulation, involved in liver dysfunction.

In conclusion, we have found that NQO1 is highly expressed in human adipocytes, and that there is a positive correlation between NQO1 expression and adipocyte size. Furthermore, NQO1 mRNA levels drastically decrease in AT during diet-induced weight loss and are highly correlated with metabolic parameters. Our findings, together with previous results from knockout studies in mice, support a link between NQO1 mRNA expression in AT and the metabolic complications of human obesity.

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